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(71) Anmelder:
BASF AG, 6700 Ludwigshafen, DE

(72) Erfinder:
Meyer, Thomas, Dr., 6700 Ludwigshafen, DE

## (54) Verfahren zur Reinigung von aktiviertem Protein C

Es wird ein Verfahren zur Reinigung von aktiviertem Protein C und analogen Proteinen durch Affinitätschromatographie beschrieben.

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5 g crude activated protein C was applied to the column with the equilibration buffer. The gel was washed with equilibration buffer until the UV absorption of the throughput remained constant and then the protein C was eluted with 0.1M glycine/HCl, 0.05M NaCl, 0.05M arginine, 0.01% Tween® 80, pH 2.5. The protein no longer contains any contaminants in accordance with SDS gel electrophoresis.

The same result is obtained if the elution is carried out with 1.75 M KSCN, 2M urea or 1.5M guanidinium hydrochloride.

Claim

A method for purifying crude activated protein C or analogous proteins, which is characterized by the fact that the crude activated protein C or analogous proteins is subjected to affinity chromatography on immobilized erythrina-trypsin inhibitor.

monoclonal antibodies to protein C were worked out recently (see Biotechnology 5, 1189 (1987)). Purification of proteins using monoclonal antibodies is very effective, but represents a significant cost factor because of the costly production of antibodies and because of the frequently unsatisfactory service life of antibody columns.

An affinity chromatographic method was developed for purification of tPA in which erythrina-trypsin inhibitor (ETI) is used (J. Biol. Chem. 259, 11635 (1984), EP-OS 112,122). Attempts to purify other enzymes like urokinase and thrombin in this way were unsuccessful.

Surprisingly, it was now found that activated protein C and analogous proteins can be purified on immobilized ETI.

The object of the invention is a method for purifying crude activated protein C or analogous proteins, which is characterized by the fact that the crude activated protein C or analogous proteins is subjected to affinity chromatography on immobilized erythrina-trypsin inhibitor.

Compounds that are analogous to protein C are to be understood to mean ones that differ from protein C by differences in the amino acid sequence or by deletions, substitutions, insertions, inversions or additions of amino acids of protein C, but have protein C activity.

"Crude protein C" is intended to mean protein C as is obtained in the processing of protein C-producing cells by means of conventional chromatographic methods. Such a protein normally has a purity of 85-95%.

The erythrina-trypsin inhibitor and its purification are known (Int. J. Biochem. 19, 601 (1987); Hoppe Seyler's Z. Physiol. Chem., 362, 531 (1981)).

The inhibitor can be coupled to all conventional affinity substrate material such as NCBr-activated sepharose, glutaraldehyde-activated tris-acyl and epoxide-activated substrate materials.

The new method has the advantage that it is simple to carry out and produces protein C of very good purity.

For this the inhibitor is coupled to the affinity substrate material so that an affinity gel results. This gel is put into a column and equilibrated with a nondenaturizing buffer. The pH of the buffer should lie between 5 and 9 and the salt concentration in the buffer should be less than 1 mol/L. After application of the crude protein it is eluted with a buffer in the weak acid range (pH 1.5-3.5).

Example 1

Erythrina-trypsin inhibitor was coupled to CNBr-activated sepharose according to the instructions of the gel manufacturer (Pharmacia). The gel (5 mL) was transferred to a column (1 cm diameter) and equilibrated with 20mM $NH_4$ acetate, 0.2M NaCl, 0.01% Tween® 80, pH 7.0.

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# A METHOD FOR PURIFYING ACTIVATED PROTEIN C

Inventor: Dr. Thomas Meyer
6700 Ludwigshafen, DE

Applicant: BASF AG
6700 Ludwigshafen, DE

[Abstract]

A method is described for purification of activated protein C and analogous proteins by affinity chromatography.

Description

The invention concerns a method for purifying activated protein C and analogous proteins.

Protein C is a vitamin K-dependent protein that occurs as a proenzyme of a serine protease. Up to now the protein was purified chiefly with the aid of chromatographic methods such as ion exchange chromatography, gel filtration, chromatography on heparin sepharose (J. Biol. Chem. 258, 1919 (1983), J. Clin. Invest. 64, 761 (1979), J. Med. 16, 285 (1985).

To simplify and to increase the efficiency of these processes, high-performance affinity chromatographic purification steps were sought and processing schemes using immobilized